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Biometry and maturation of *Sesbania punicea* (Cav.) Benth. seeds

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Abstract: The objective of the study was to evaluate the biometry of fruits and seeds of *S. punicea* in order to determine the point of collection for production of seedlings of the species. The fruits of *S. punicea* were collected from plants *in situ* in Barra do Ribeiro, RS. In the laboratory, they were separated by staining in: stage 1, 2 and 3. Fruits and seeds were measured with digital caliper (mm), counting the number of seeds and locules. At each stage water content, seed dry matter and germination test were evaluated. For this last one, four replications of 25 seeds were placed in germination chamber during 16 days, photoperiod of 12 hours and temperature of 25ºC. The variables analyzed were germination, germination speed index, average germination time, percentage of normal and abnormal seedlings, and hard seeds. It was observed that in stage 2 the seeds have larger dimensions, a higher percentage of germination and formation of normal seedlings, and a higher rate of germination speed. It is concluded that fruits must be harvested at stage 2 of maturation, as seeds have the highest rates of germination and normal seedlings, as well as the lowest rates of insect damage.

Key-words: Morphological characterization, germination seeds, native plant.

Biometria e maturação de sementes de *Sesbania punicea* (Cav.) Benth

Resumo: O objetivo do trabalho foi avaliar a biometria de frutos e sementes de *S. punicea* a fim de determinar o ponto de coleta para produção de mudas da espécie. Os frutos de *S. punicea* foram coletados em plantas *in situ* em Barra do Ribeiro, RS. No laboratório, foram separados pela coloração em: estádio 1, 2 e 3. Os frutos e as sementes foram mensurados com paquímetro digital (mm), contados o número de sementes e lóculos. Em cada estádio foi avaliado o teor de água, matéria seca de sementes e teste de germinação. Para este último utilizou-se quatro repetições de 25 sementes acondicionadas em câmara de germinação durante 16 dias, fotoperíodo de 12 horas e temperatura de 25ºC. As variáveis analisadas foram germinação, índice de velocidade de germinação, tempo médio de germinação, porcentagem de plântulas normais e anormais, e sementes duras. Foi observado que no estádio 2 as sementes apresentam maiores dimensões, maior porcentagem de germinação e formação de plântulas normais, e maior índice de velocidade de germinação. Conclui-se que os frutos devem ser colhidos no estágio 2 de maturação, quando as sementes apresentam maiores taxas de germinação e formação de plântulas normais, bem como, menor taxa de danos por inseto.

Palavras-chave: Caracterização morfológica, germinação de sementes, planta nativa.

Introduction

Sesbania punicea (Cav.) Benth. commonly known as scarlet wisteria, red sesbania, or rattlebox, is a 2-4 meter high, deciduous tree that belongs to the Fabaceae family. It has potential as ornamental plant, mainly due to the orange color and raceme-shaped inflorescences. Leaves are alternate with numerous leaflets (Stumpf et al., 2009; Queiroz, 2020). Native to the Pampa biome, it also occurs on the coast of Argentina and Uruguay (Izaguirre and Beyhaut, 1998).

This species has already been cultivated as an ornamental plant in South Africa and the United States (Csurhes and Edwards, 1998), however, in the literature, there is little information related to specific aspects of flowering and fruit maturation, which is fundamental information for propagation, and consequently, the use as ornamental plant.

Seed maturation is genetically controlled, involving a series of transformations from the fertilization to the moment when the seeds become independent from the original plant (Marcos-Filho, 2015). Therefore, physiological maturity is reached when the seeds reach maximum germinative potential and vigor and can define the harvest point, which is determined, among other factors, by environmental conditions (Carvalho and Nakagawa, 2000; Gemaque et al., 2002).

Plant species, in general, lack uniformity in development or maturation of fruits and seeds. This is because inflorescences are not pollinated in the same period (Marcos Filho, 2015). In *S. punicea*, it is possible to observe uneven flower opening on different parts of the plant, therefore, the same plant will show fruits in different stages of maturation.

The morphological characterization of fruits and seeds aims at species identification and knowledge on ecological aspects of the plant such as dispersion, seedling establishment, and ecological succession (Matheus and Lopes, 2007). Seed size can influence vigor. Larger seeds will produce more vigorous seedlings, which can be positive in establishing crops (Carvalho and Nakagawa, 2000).

Seed germination is a physiological process for species perpetuation and for agriculture in general. This phenomenon can be affected by several environmental factors, including temperature and water (Marcos Filho, 2015). Other external factors can also affect germination, e.g., the presence of diseases or insects.

Studies related to insect predation in plant species are important because they have a direct influence on the ability of plants to propagate (Grenha et al., 2008). *S. punicea* is attacked by the small beetle *Rhyssomatus marginatus*, which in the larval stage feed on the seeds. Each larvae can feed on up to three seeds and on average they can decrease 88% of the available seeds (Hoffmann and Moran, 1992).

In general, few studies in the literature report on phenology and later aspects related to the germination and characterization of fruits and seeds of native plants, including the species *S.*

punicea. Therefore, research on this theme is important to understand the behavior of the species with regard to time of seed physiological maturity, insect predation rate, and biometric data of fruits and seeds, since these factors can influence the correct time of seed collection, establishment and growth of seedlings.

The objective of the study was to evaluate the biometry of fruits and seeds of *S. punicea* in order to determine the point of collection for production of production of seedlings of the species.

Material and methods

The fruits of *Sesbania punicea* were collected from approximately 20 trees, in Barra do Ribeiro-RS, (30°21'27"S 51°20'32"W") and packed in plastic bags for transport to the laboratory, on February 22, 2016. Specimens were registered in the Herbarium with the identification number IN185165.

After collection, 10 fruits were separated by visual evaluation into stages according to color: stage 1 (green fruits), stage 2 (yellow fruits); and stage 3 (brown fruits). Fruit color (stages 1, 2, and 3) was evaluated one day after collection (23/02/2016), with a Minolta CR-400 colorimeter, using the CIELab system to evaluate the parameters “L*”, “a*”, and “b*”: where L * is the brightness ranging from 0 (black) to 100 (white) and a* and b* the chroma coordinates (-a* = green, + a* = red, - b* = blue and + b* = yellow), both ranging from -60 to +60 (Minolta, 1993).

From a random sample of 100 fruits and seeds of each stage (1, 2, and 3), biometric assessments determining the width, length, and thickness (median part of the fruit and seed) were performed with a digital caliper and expressed as mm. Number of locules, intact seeds and seeds damaged by insects (showing exit hole made by insect) were also counted.

After fruit screening, water content and dry matter of seeds were evaluated, using four repetitions of 1 g for each stage (1, 2, and 3). Fruits were weighed on a semi-analytical scale (0.01g), placed in an oven at 105 ºC for 24 h, and weighed again (Brasil, 2009).

For the germination test, the intact seeds (without insect attack) were disinfested with 1% sodium hypochlorite for 3 minutes and triple washing with distilled water for 1 min. Subsequently, the seeds were wrapped in two sheets of autoclaved germitest paper and moistened with distilled water 2.5 times the weight of the sheet. Four repetitions of 25 seeds were used for each stage (fruit color: stage 1 - green fruits, stage 2 - yellow fruits and stage 3 - brown fruits). The seeds were kept in a germination chamber with a photoperiod of 12 hours and 25ºC of temperature, for 16 days.

The variables evaluated were: physiological germination (radicle protrusion) (G%), normal seedlings (NS%), abnormal seedlings (AS%), hardseededness (non-germinated) (HS%), germination speed index (GSI), which was calculated according to Maguire, 1962, modified by Santana and Ranal, 2004, and the average germination time (AGT) was evaluated (Silva and Nakagawa 1995).

Seedlings were considered as normal when presenting all the essential structures for the development of the future plant such as primary and secondary roots, while abnormal seedlings presented defects and were incapable of developing into a normal plant, and seeds that did not germinate were classified as hard seeds (Brasil, 2009).

The software applications used to analyze the data were SigmaPlot 11.0 and Costat. The characterizations of fruits and seeds were analyzed by descriptive analysis and analysis of variance, and means were compared by the Tukey's test at 5% probability of error. Variable data that did not meet the assumptions of normality were transformed. The variable normal seedlings was transformed to $\sqrt{(x)}$ and hard seeds to x/5. However, the variable abnormal seedling remained not normally distributed after transformation, thus non-parametric analysis and the Kruskal Wallis test were used.

Results and discussion

Analysis of the coordinates a*, b*, and L* showed color varied according to the stage of *S. punicea* fruits: at stage 1 the fruits were characterized in the Cielab space as light green, very close to the yellow; at stage 2, the fruits were characterized as yellow; and as brown at stage 3 (Table 1 and Figure 1).

In a study of maturation stages of *Jatropha curcas* L., the authors identified, using a digital color analyzer, a predominantly yellow color in the fruit epicarp at stages 2 and 3 and a decrease in the readings of the refraction scale from the stages 5 and 6 (Dranski et al., 2010). These characteristics were similar to those observed in this study, in which the colorimetric analysis demonstrated differences in fruit color (Table 1).

Table 1. Averages of variables (L *, a *, b *), of stages 1, 2 and 3 of fruits of *Sesbania punicea* (Cav.) Benth., collected in Barra do Ribeiro, RS

	Stage 1			Stage 2			Stage 3		
	Staining variables								
Date	L*	a*	b*	L*	a*	b*	L*	a*	b*
23/02/16	51.58	-6.60	26.50	56.05	0.11	26.41	36.41	3.33	6.79

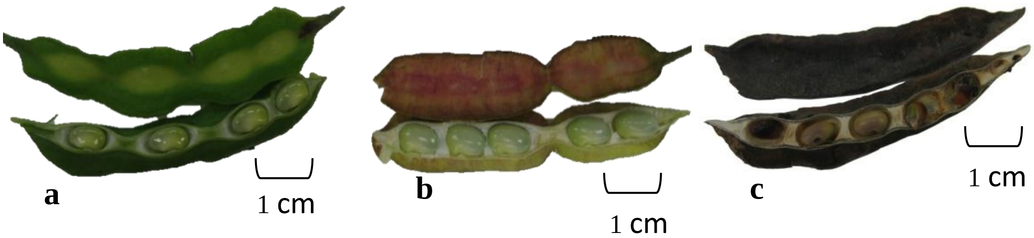

Figure 1. Fruits of *Sesbania punicea* (Cav.) Benth. at the stage 1 (a), 2 (b) and 3 (c).

Difference was found for fruit width between stages 2 and 3 and for thickness between stages 1 and 3, but no difference was found for fruit length at the stages evaluated (Table 2), showing that the different stages influenced thickness and width, but not length of fruits. The results of this study for width and length of fruits agree with Izaguirre and Beyhaut (1998), who described fruits of this species measuring 45 - 100 mm in length and 12 - 23 mm in width. Table 3 shows the averages, minimum, maximum and standard deviation of fruit measurements.

Seed size differed between stages, being that in the stage 2 seeds were the largest, especially in width and thickness, reaching 6-7.5 mm in width, which was close to values reported by Burkart (1987). No difference was found for the number of locules and number of seeds, fruits having in average 5.98 locules and seeds (Table 2).

When plants allocate more reserves to seeds, an increase occurs in seed size, mainly in thickness, reaching the maximum point near the half of the period of dry matter accumulation. Subsequently, the decrease in water content, especially in legumes, causes a decrease in size (Marcos Filho, 2015). The present study also identified a reduction in seed size, in which *S. punicea* seeds increased in size from stage 1 to stage 2, and then decreased in stage 3, demonstrating the accumulation of dry matter in the seeds and a decrease in the content of water with maturation, respectively.

The number of seeds attacked by insects of fruits at stage 3 corresponded to 50.4%, which reduced the number of seeds available for germination, while seeds of fruits at stages 1 and 2 had low rate of damaged seeds, 2.9% and 11.9% respectively (Table 2).

In a study with *Erythrina crista-galli*, belonging to the Fabaceae family as well, Mello et al. (2013) found a large number (71.8%) of seeds damaged by insects, mainly *Pityophthorus* sp, from the Family Curculionidae, subfamily Scolytinae.

Table 2. Average values of length (LF), width (WF), fruit thickness in mm (TF), length (LS), width (WS), seed thickness in mm (TS), number of locules (Nº. L), number of total seeds (Nº. TS) and seeds damaged by insects (% SDA) of *Sesbania punicea* in three stages

Fruit	LF	WF	TF	LS	WS	TS	Nº L	Nº TS	SDA%
Stage 1	71.^{ns}	8.7 ab*	9.0 b	6.64 b	4.53 b	3.07 c	6.27 ^{ns}	6.27 ^{ns}	2.9 a
Stage 2	67.5	8.18 b	11.4 a	8.11 a	5.65 a	4.19 a	5.54	5.54	11.9 a
Stage 3	67.2	9.33 a	9.22 b	5.66 c	4.23 c	3.62 b	6.15	6.15	50.4b
Mean	68.8	-	-	-	-	-	5.98	5.98	-
P-value	0.15	0.01	< 0.01	< 0.01	< 0.01	< 0.01	0.10	0.10	<0.01
C.V.(%)	4.71	5.49	5.63	1.3	1.91	1.84	7.54	7.5	30.47

* Means followed by different letters in the column differ by Tukey's test at the level of 5% probability of error. ns = not significant.

Because *S. punicea* is a native plant without a well-established cultivation technology, no products have been registered to control *R. marginatus* in this species, however one of the methods of control of this insect is the growing of *S. punicea* mother trees in protected environments to prevent insects from accessing the plants, as it happens in the natural environment.

The water content of stage-1-fruit seeds was the highest (72.13%), followed by stage 2 (58.58%), and stage 3 (9.83%) with the lowest content. However, the variable seed dry matter showed an opposite result, with 18.39, 47.91, and 51.54% for stages 1, 2, and 3, respectively (Table 4). A similar result was found by Aquino et al. (2006) for Yellow Poinciana ((*Peltophorum dubium* (Spreng.) Taub.), with water content of 61.96% at stage 1 and 11.88% at stage 4. Similar result was found for *Jatropha curcas*, with the water content in the seeds decreasing with fruit ripening, reducing to 14.48% at the dry fruit stage in the beginning of dehiscence (Dranski et al., 2010).

Seeds reach maximum dry matter when the water content is between 35% and 55% (Marcos Filho, 2015). Based on this information and the data recorded in this work, the maximum accumulation of dry mass of *S. punicea* seeds possibly occurred between stages 2 and 3.

Table 3. Average values, minimum value (Min. V.), average, maximum value (Max. V.), standard deviation (SD) and coefficient of variation (C.V.%) of length (L), width (W), thickness (T) in mm of fruits and seeds and number of locules (Nº. L), of *Sesbania punicea* (Cav.) Benth. in three stages 1, 2 and 3

	Color	Variables	Min V.	Mean	Max V.	SD	C.V.(%)
Fruits	Estage 1	L (mm)	31.7	71.7	91.1	9.2	12.9
		W (mm)	5.2	8.6	13.2	1.6	18.4
		T(mm)	5.2	9.0	13.8	2.0	22.2
		Nº Lóc	3.0	6.3	9.0	1.3	20.6
	Estage 2	L (mm)	42.1	67.5	96.8	10.9	16.2
		W (mm)	6.3	8.2	13.2	1.2	14.6
		T (mm)	7.7	11.4	13.6	1.1	9.9
		Nº Lóc	1.51	5.5	2.0	8.0	27.3
	Estage 3	L (mm)	50.6	67.8	87.9	7.6	11.3
		W (mm)	6.2	9.3	12.4	1.1	11.3
		T (mm)	5.8	9.2	13.1	1.4	15.4
Seeds		Nº Lóc	3.0	6.1	8.0	1.1	19.1
	Estage 1	L (mm)	5.5	6.6	8.3	0.5	7.7
		W (mm)	3.4	4.5	5.6	0.3	7.8
		T (mm)	2.3	3.0	4.0	0.3	11.1
	Estage 2	L (mm)	7.0	8.1	9.0	0.4	5.1
		W (mm)	4.2	5.6	6.4	0.4	7.2
		T (mm)	3.2	4.1	8.7	0.5	12.7
	Estage 3	L (mm)	4.6	5.66	6.8	0.4	7.3
		W (mm)	3.5	4.22	4.9	0.3	7.3
		T (mm)	3.0	3.60	5.0	0.2	8.1

The highest germination rate (G%) was recorded for stage 2-fruit seeds, reaching 86%, whereas stage-1-fruit seeds 1 and stage-3-fruit seeds had germination below 10% (Table 4).

The stage-1-fruit seeds were not suitable for germination, showing a low radicle protrusion index. Right after the fertilization of the ovule, the seeds of some species have the ability to grow the radicle, however normal seedlings are not formed, which requires a complete accumulation of reserves and histodifferentiation (Marcos Filho, 2015). In *Erythrina crista-galli* L., higher germination rates were found in the first weeks after collection, that is, 7 and 8 weeks after anthesis. However, from weeks 9 and 10 there was a decrease in germination. The authors reported that seed dormancy began from weeks 9 and 10 after the collection (Lazarotto et al., 2011).

In *Piptadenia viridiflora* (Kunth) Benth., Pessoa et al. (2010) evaluated different seed maturation stages and found low germination and 33-63% of hard seeds. The authors discussed that this result was due to seed coat impermeability. However, these findings differed from those obtained for *Poincianella pluviosa* in a test with 14 maturation stages, in which the rate of germination or protrusion of the radicle increased from stage 6 to stage 7. Fruit color was green with brown spots, and the peak of germination was reached at the stage 13 (brown fruits), with about 90% of germination rate (Silva et al., 2015).

Table 4. Average values of water content (WC), dry matter of seeds (DMS), germination (% G), germination speed index (GSI), mean germination time (MGT), normal seedlings (% NS), abnormal seedlings (% AS), hard seeds (% HS) in three stages of fruit ripening of *Sesbania punicea* (Cav.) Benth.

Variables	Stage 1	Stage 2	Stage 3	P-value	CV (%)
WC (%)	72.13 a*	58,58 b	9,83 c	< 0.01	1,28
DMS (mg/seed)	18.39 c	47.91 b	51.54 a	< 0.01	1.41
% G	9.0 b	86.0 a	9.0 b	< 0.01	21.24
GSI	0.14 b	0.21 a	0.11 b	< 0.03	12.52
MGT (days)	7.07 ab	5.47 a	8.92 b	< 0.94	17.60
% NS	4 c	66 a	8 b	< 0.01	21.9
% AS	0 a	10 b	0 a	< 0.14	89.4
% HS	91 b	14 a	91 b	< 0.01	11.50

* Means followed by different letters on the line differ from each other by the Tukey test at the level of 5% probability of error.

In this study, the seeds from stage 2 also performed better for germination speed index (GSI), differing from the other treatments. This result differed from *Jatropha curcas* (Euphorbiaceae), which showed the highest GSI of seeds from stage 5 fruits (ripe fruits) and stage 6 fruits (dry fruits at the beginning of dehiscence). Seeds from green fruits did not germinate and GSI could not be determined (Dranski et al., 2010).

Were also observed significant difference in the data of mean germination time (MGT) between the seeds from fruits at stages 2 and 3, but the seeds at these two stages were not different from stage 1 seeds. This result is similar to what occurred in the study of seed maturation of *Eugenia involucrata*, a species belonging to the Myrtaceae family. Seeds from green fruits took longer time to germinate, differing statistically from seeds from light red fruits. However, they did not differ significantly from seeds of yellow/redish, burgundy, and fruits fallen on ground (Oro et al., 2012).

The rate of normal seedlings was 4% in stage-1-fruit seeds, while in stage-2-fruit seeds was 66%, and of stage-2-fruit seeds was 8%. Lazarotto et al. (2011) obtained similar results for seeds of *Erythrina crista-galli*, with 50% of normal seedlings recorded at week 8 after anthesis, which is identified as the point of seed physiological maturity. In abnormal seedlings, the highest rates of normal seedlings (10%) were found in stage-2-fruit seeds. No abnormal seedlings were recorded at other stages of maturation because there was less seedlings formed. This result differed from that found in the study of physiological maturity of seeds of *Erythrina crista-galli* by Lazarotto et al. (2011), who found 25% of abnormal seedlings in the week 7 after anthesis.

Stage-2-fruit seeds showed 14% of hardseededness, and both treatments, stage 1 and stage 3, had 91%. The results related to germination and percentage of hardseededness are likely to be due to the physiological immaturity of the stage-1-fruit seeds, which are not able to germinate. Stage-

3-fruit seeds, on the other hand, possibly entered into seed coat dormancy as verified by Graaff and Van Standen (1983), who found that seeds of the Fabaceae family presents coat-imposed dormancy. In this case, physical, thermal, or chemical treatments should be carried out overcome the dormancy of seeds of this species for a greater uniformity and percentage of germination.

The stage-2-fruit seeds proved to be the most suitable among the treatments evaluated for immediate sowing and, thus, start seedling production, as they had the highest germination index and the lowest percentage of insect-damaged seeds compared with the stage-3-fruit seeds. Seeds at stage 1 of maturation showed the lowest results for most germination variables, as they are not physiologically mature, besides, the water content at 72% makes it difficult to store.

Conclusion

S. punicea fruits are on average 8.74 mm wide, 68.8 mm long, and 9.86 mm thick. Each fruit has on average 5.98 locules and seeds, which are 6.74 mm long, 4.80 mm wide, and 3.63 mm thick on average. Fruits must be harvested at stage 2 of maturation, as seeds will have the highest rates of germination and normal seedlings, as well as the lowest rates of insect damage.

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